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ORIGINAL ARTICLE

EFFECT OF CADMIUM CHLORIDE ON HISTOLOGICAL CHANGES IN THE GILL TISSUE OF *Labeo rohita*

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ABSTRACT

The present study is aimed to observe the histopathologcal changes in the gill tissue of fresh water fish, Labeo rohita exposed to sublethal concentration of cadmium chloride.The histopathological changes were observed in the gill tissues of *Labeo rohita* after exposure with cadmium chloride. The present study shows that necrosis of secondary lamella, damaged secondary lamellae and the secondary lamellae in the cadmium chloride exposed fish

Keywords: Cadmium Chloride, Histology, Gill, *Labeo rohita*

1.INTRODUCTION

Heavy metal constitute a serious type of pollution in fresh water and being stable compounds, they are not readily removed by oxidation, precipitation or other processes and affect the activity in recipient animal (Nammalwar, 1985). Heavy metals such as chromium, mercury, lead and arsenic are non essential elements and are toxic to the aquatic organisms even at low levels. The cumulative concentration of pollutants along the food chain poses a threat to both human and animal health.

Heavy metals are natural trace components of the aquatic environment, but background levels in the environment have increased especially in areas where industrial, agricultural and mining activities are widespread (Bryan and Langston, 1992; Langston, 1990). Most of the heavy metals released into the environment, find their way into the aquatic phase as a result of direct input, atmospheric deposition and erosion due to rain water. Therefore, aquatic animals may be exposed to elevated levels of heavy metals due to their wide use for anthropogenic purpose.

Heavy metal contamination has been reported in aquatic organisms (Rashed, 2001; Adham *et al.*, 1999). These metals build up in the food chain and are responsible for chronic illness and death in aquatic organisms (Farkas *et al.*, 2002). Abel (1989) has found that lead, mercury, zinc, copper and cadmium are the important heavy metals which pollute the water.

The health of fish may be affected, either directly through uptake from the water, or indirectly through their diet of vegetation, invertebrates or smaller fish. Metals released into aquatic ecosystems, are responsible for several fish physiology irregularities (Sehgal and Saxena, 1986). They can also disturb the ionoregulatory mechanism in aquatic organisms (Hansen *et al.*, 1996). All of these effects of heavy metals usually affect fish negatively leading to stress and eventually, in most cases, death.

Fishes are sensitive to contaminants of the water and pollutants may damage certain physiological and biochemical processes when they enter the organs of the fish(Tulasi *et al.*,1992). The heavy metal in the tissue of fishes may cause various physiological defects and mortality (Torres *et al.*,1987). The fishes which are largely being used for the assessment of the quality of the aquatic environment and can cause bioindicator of environmental pollution (Dautrempuits et al. 2004; Lopes *et al.*,2001).Fish gills, which serve as the primary uptake site in fish for trace metals, represent the most important targets when exposed to elevated levels of ambient metals (Newman and Jagoe, 1994). Gills are the vital organs for respiration of fish, which establish a direct contact with the medium through which the pollutants largely enter into the body (Mount 1962; Holden 1972; Edward 1973). The gills are the first organs to be affected by the toxicants among the other organs due to fish nature (Fylizer, 1978). The gills

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serve as the most sensitive index to monitor environmental alterations (Mahajan and Singh, 1973).

2. MATERILAS AND METHODS

Procurement of experimental animal

The fresh water fish *Labeo rohita* were collected from the fish farm located in Puthur, Nagapattinam District, 15 Km away from the Uinversity campus. This fishes were brought to the laboratory and transferred to the rectangular fibre glass tanka (100X175cm) of 500 liters capacity containing chlorine free aerated wellwater.

Acclimatization of animals

The fresh water fish, *Labeo rohita* were limatized for a minimum period of 15 days in the laboratory conditions at room temperature (28±1ºC) before subjecting them for screening test. These fingerlings were fed with artificial food pellets on alternative days and the water renewed every 24 hours. The tanks were rinsed with potassium permanganate or acroflavine (2mg/l) to prevent fungal attack.the fresh water fish, *Labeo rohita* were critically screened for the signs of disease, stress, physical damage and mortality./the injured, severely diseased, abnormal and dead fishes were discarded. The feeding was discontinued 24 hours before the beginning of the experiment to reduce the excretory products in the test trough as suggested by Arrora *et al*., (1972). During the acclimation, the fishes were reared in tank until there was less than 10 percent mortality in 4 days perior to the beginning of the test as suggested by Anderson (1977). The water in the experimental trough was changed daily and also aeration was stopped to avoid the possible oxidation of the toxicants.

Selection of Cadmium Chloride

The toxicant, cadmium chloride was used for the present experimental studies. It is rarely found in pure state, it is present in various types of rocks and soils and in water as well as in coal and petroleum. The sulfate, nitrate and halides are soluble in water. Cadmium (atomic number 48, relative atomic mass 112.40) is a metallic element was used for the present study. It is rarely found in pure state, it is present in various types of rocks and soils and in water as well as in coal and petroleum. Among these natural sources, zinc, lead, copper is the main sources of cadmium. Its mobility in the environment and effects on the ecosystem depend to a great extent on the nature of these salts. Some cadmium salts, such as the sulfide, carbonate and oxides are insoluble in water. The sulfate, nitrate and halides are soluble in water. The speciation of cadmium in the environment is of importance in evaluating the potential hazards. In the present investigation cadmium chloride has been selected for the present experimental study. The physical and chemical properties of cadmium chloride are given below.

Toxicity Studies

To evaluate the acute toxicity studies the static renewal toxicity test were conducted according to the methods recommended by American Public Health Association (1960). In the present investigation the toxicity of cadmium chloride (LC_{50}) for 96 hours were analyzed. The LC_{50} is statistical estimate to the concentration of toxic material in water that kills 50 per cent of the test species, under experimental conditions during a specific time interval. The LC_{50} was used because the concentration required to affect the response in 50 percent of the test animals is more reproducible than any other value (Pickering and Handerson, 1966).

Screening test

The screening test was conducted to avoid delay and to save time and effort. The object of this test is to obtain approximate indication of the concentration of a substance likely to be hazardous to the test fish and fishes in general in their natural environment.

The toxicant concentration used in the present series of tests were approximately the wide range of concentration viz., 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 ppm aqueous solutions were prepared. The tests were conducted in the rectangular plastic troughs. The troughs were cleaned well and dried before conducting experiments. Then the tests were conducted by allowing ten fishes of *Labeo rohita* in each plastic trough containing 10 liters of water with particular concentration of the cadmium chloride. The screening tests was continued to assess the concentration at which all fishes survived for 24 hours and likewise the concentration at which most of the fishes died simultaneously (Bansal *et al.*, 1980).

Definitive test

Preliminary observation showed that beyond 30 ppm of cadmium chloride all the test fishes died. Therefore the concentration of cadmium chloride falling of within 1 to 30 ppm were prepared and ten number of test fishes were introduced to confined narrow range of concentration viz., 1,2,3,4,5,6,7,8,9,10 ppm of cadmium chloride solutions. The behavioral responses of the fish at various concentration of cadmium chloride were observed at regular intervals to ascertain the impact of the cadmium toxicity on the organism. Individuals in the test medium, which showed no responses to stimulation and those without opercular movement, were removed quickly to avoid cannibalism among the fish. In all tests, mortalities were recorded 96 hours.

The LC_{50} values were determined by following the method of Finney (1971). Sublethal studies are helpful to assess the response of the test organisms under augmented stress caused by metals. 96 hr LC_{50} value for cadmium chloride was found at 1.87 ppm. Hence the one tenth of 96 hr LC_{50} value (1.87 ppm) was selected for the present investigation as sublethal concentration.

Experimental design

The toxicant exposure was done by 24 hour or renewal bioassay system. For analysis sublethal toxicity, 3 groups of 10 fish each were exposed separately and cadmium chloride (8.5ppm: 10 % 96 hours LC_{50}). Solution prepared in well water. The experimental medium was prepared by dissolving cadmium chloride at 1.87 ppm having dissolved oxygen 5.8 ppm, pH7.4, water hardness 30.3mg/l (APHA *et al*., 1992)

and water temperature 28± 2 C. Each group was exposed to 50 l of the experimental medium. Parallel groups of 10 fish each were kept in separate aquaric containing 50 l of well water as control. Feeding was allowed in the experimental as well as control groups every day for a period of 3 hours before the reneval of the medium throughout the tenature of the experiment.

The experimental fish were exposed to sublethal concentration (1.87 ppm) of cadmium chloride for a period of 14 days at intervals of 7and 14 days. The control and experimental fish were dissected out at the end of each period of exposure and the selected organs such as gill, liver and kidney were dissected out for bioaccumulation. The blood samples were also collected for haematological parameters. The tissues later were processed for histological and histopathological studies.

Validity of Analytical Procedures

Fishes of the same size and weight were used throughout the study. Well water whose characteristics did not change noticeably during the course of study was used. The dissolved oxygen level of the experimental solutions was estimated regularly and an optimum dissolved oxygen level was maintained in all the studies. Test solution was renewed every 24 hours to maintain the same cadmium concentration level during later hours of exposure. The experimental solutions were all prepared just prior to the test. For the experimental analysis the fresh water fish *Labeo rohita* were divided into two groups. One group of fish were maintained as to control medium and another group of fish were exposed to sublethal concentration of cadmium chloride solution,

Histological preparations

For histological studies the test organs were dissected out from the treated and control fish and fixed quickly in Bouin's fluid. After 24 hours of exposure the gill, liver, intestine and kidney were processed by following the standard techniques (Gurr, 1959). The gills were treated with 5 percent nitric acid for 24 hours to soften the cartilage before processing. After dehydration in alcoholic series, the tissues were transferred into absolute alcohol and acetone for completing dehydration and later into xylol for clearing till the material become transparent. The tissues were embedded in paraffin wax (E'merck 58 – 60^{0}C). Sections were cut at 6 µ thickness and deparaffinised sections were stained in Heidenhains alum-Haematoxylin and counterstained with aqueous eosin for microscopic observations.

3.RESULTS

Histology of control gill

The gills consist of an arched bar, which carries two primary lamellae (PL) which are elongated and flattened arising along with its length. Plate I- 1,2 shows the Low magnification of control gill tissues of *Labeo rohita.* The Secondary Lamellae (SL) arising from the primary lamellae and alternating with each other. The respiratory supplied by the secondary lamellae which use involved in the respiration process. When the secondary lamellae compared with primary lamellae, it is very thin. The primary lamellae are lined with intra lamellar epithelium, which are closely arranged to the gill ray (GL) which runs in the primary lamellae centre. The Plate I- Fig.2 shows the higher magnification gill of control fish. The primary lamellae and secondary lamellae are uniformly arranged.

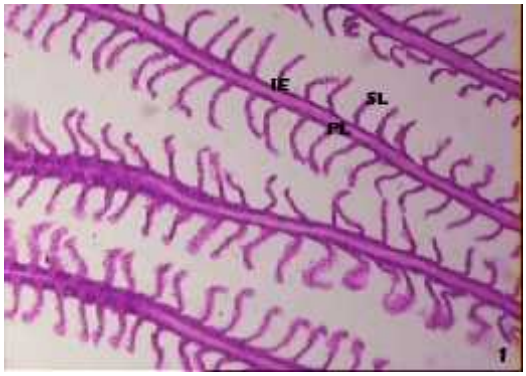

Fig.1.Section of the gill of control fish,*Labeo rohita* showing interlamellar;epithelium, primary lamellae and secondary lamellae. IE– Interlamellar epithelium;PL- Primary lamellae;SL- Secondary Lamellae

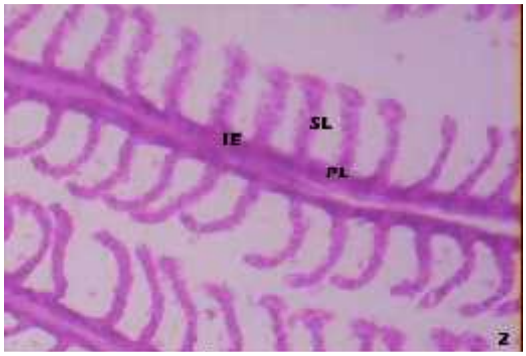

Fig. 2. Enlarged portion of Fig.1 showing the secondary lamellae, Respiratory epitheliem and chloride secretory cells.SL- Secondary Lamellae;RE- Respiratory epitheliem

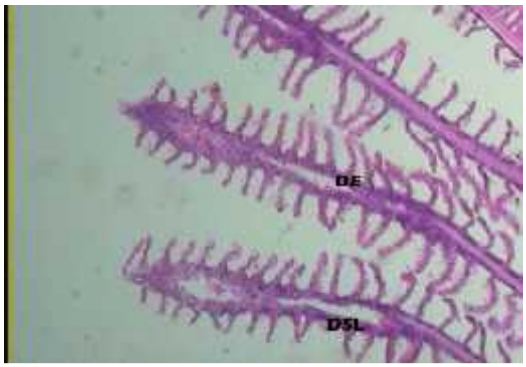

Fig. 3.Section of the gill of *Labeo rohita* exposed to cadmium chloride showing degenerated respiratory epithelium, Mucous covering, damaged secondary lamellae and enlarged secondary lamellae. DE- Damaged Epithelium;;MC- Mucous covering;DSL- Damaged secondary lamellae

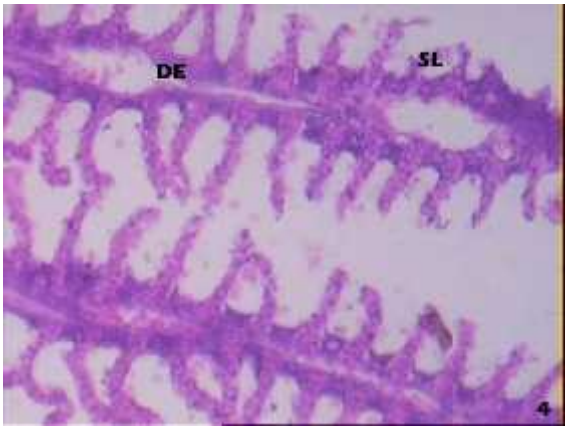

Fig.4.Enlarged portion of Fig.3 showing vacuoles, mucous coveringand damaged secondary lamellaeV – Vacuoles;DSL- Damaged secondary lamellae

Histopathology of treated gill

Plate I shows the gill of cadmium exposed fish. The sub-lethal concentration of cadmium chloride shows the damaged histoarchitecture of gill. The primary lamellae (PL) and secondary lamellae (SL) are ruptured and not uniformed in all regions. The primary lamellae were broken down in some places. The gill epithelium shows the extensive thickening and hyper plastic lesions. Due to cadmium intoxication the gill rays (GR) separated the epithelium and secondary Lamellae and formed lamellae and interlamellar epithelium. The secondary lamellae tips are very narrow and short by the administration of cadmium chloride. The basement membrane is destructed in some places. The mucous substances covering the two primary lamellae and secondary lamellae in some places. The vacuolization formed in certain area of secondary lamellae. Plate I- 3,4 shows the higher magnification of gill tissues of fish *Labeo rohita* exposed to sub-lethal concentration of cadmium. This figure show that the marked deterioration of respiratory epithelial cells. The mucous substances covering around the interlamellar spaces of the gill. The extensive hyperplastic lesions are seen in the gill epithelium. The efferent blood vessels are damaged at the proximal region. The curling and clubbing at tips of the secondary lamellae are noticed in Plate I- 3,4 The pillar cells and respiratory epithelial cells are destructed in the secondary lamellae due to the toxicity of cadmium.

4.DISCUSSION

The histopathological studies on fish is a note-worthy and promising field to understand the extent to which changes in the structural organization occurs in the organ due to pollutants in the environment. At microscopic level, the cellular organelles lead to alterations in functional systems. Rana *et al*., (1982) have stated that the histopathological changes are irreversible while; altered functional system is considered as a reversible effect. Vijayamadhavan and Iwai (1979) have reported that the extent of damage varies with organs, nature of pollutants, medium and test duration. The mortality at fishes occurs due to the pathological lesions caused by mercury. Further critical studies on the histopathological effects at metals on fishes may help to establish the specificity between the metal and their effects.

The various studies have shown that histopathological changes occur in gills of fishes exposed to heavy metals such as inorganic mercury in Rainbow trout (Daoust *et al*., 1984; Khangarot *et al*., 1980), cadmium chloride in *Tautogoloborous adspersus* (Newman and Macloan, 1974), Lead in *Barbus stigma* (Natarajan, 1979) and Methyl mercuric chloride in *Channa punctatus* (Sastry and Rao, 1982).The histopathological changes noticed in the gills of *Labeo rohita* and due to the sensitivity of gill tissue and also due to the exposure of the respiratory surface to cadmium. The freshwater fish *Labeo rohita* showed the histopathological changes treated with sub-lethal concentration for 7 and 14 days. Thickening of intralamellar epithelium, reduction in the interlamellar space, loss of epithelial cells, destruction at the basement membrane and erosion of primary lamellae are observed. The similar studies were made by Ashok Kumar Gupta and Vinod Kumar Rajbanshi; (1995) in Rasbora deniconius (Hamilton) exposed to mercury. In the present study, the gill tissues show the destruction and thinning of interlamellar epithelium, which may be due to destruction and disappearance of the interlamellar cells. Santhakumari (1990) has also observed the destruction and disappearance of interlamellar cells leading to the thinning of epithelium in the gill tissue of *Anabus testidineus* exposed to mercury.

The loss of respiratory epithelium and the formation of haemotomass within the secondary lamellae might be lead to a great reduction of the respiratory surface resulting in the impairment of oxygen uptake to a considerable extent, moreover, heavy metal ions in the medium precipitate, mucous secretions produced by the gills. The interlamellar spaces become filled with this precipitate, inhibiting the normal movement of the gill filaments.

5. REFERENCES

Abel, P.D, 1989. *Water Pollution Biology*. Ellis Horwood Publishers, Chichester. 231pp.

Anderson, R.L. 1977. Chronic toxicity tests – Biological back – ground and procedure. American Soc. For testing and materials, Blacksburg, VA. 27-29.

APHA, American public heath association, AWWP, American water association are w PGF, water pollution and fareatru 1997. standad methods for the examination of water and waste water. 19th eds. Washington. DC.

Arrora, H.C., V.P. Sharma, S.N.Chattopathya and L.P.Sinha, 1972. Bioassay studies of some commercial organic insecticides part-III. Trials of *Cirrhinus mirgala* with 6 insecticides. *Indian J.Environ. Hlth.,* 14(4): 352-359.

Ashok Kumar Gupta and Vinod Kumar Rajkanshi, 1995. Mercury poisoning, architectural changes in the gill of *Rasbora daniconius* (hamilton). *J. Environ. Biol*., 16 (1): 33-36.

Bansal, S.K., S.R.Vermam.,A.K.Gupta and R.C.Dalela, 1980. Predicting long term toxicity by sub-acute screening of pesticides with larval and early juveniles of four species of freshwater major carp. *Eco. Environ. Saf.,* 4(3):224-231.

Bryan, G. and Langston, W.J. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. *Environmental Pollution* 76, 89–131.

Daoust, R.V., wobeser, G., and New Stead, J.P. 1984. Acute pathological effects of inorganic mercury and copper in gill of raninbow trout. *Vet. Pathol.* 21(1) 92-101.

Dautremepuits, C., Paris-Palacios, S., Betoulle, S., and G. Vernet, 2004. Modulation in hepatic and head kidney parameters of carp (*Cyprinus carpio* L.) induced by copper and chitosan. *Comp. Biochem. Physiol.,* **137**: 325-33.

Farkas, A., Salanki, J. and Specziar, A. 2002. Relation between growth and populating metal concentration in organs of bream, *Abramis brama* L. populating lake Balation. *Arch. of Environ, contam, and Toxicol.* 43 (2): 236-243.

Finney, DJ, (1971). Probit Analysis. Cambridge University Press, London, p. 333.

Gurr, E. 1959. Methods of analytical Histology and Histochemsitry. Leobard Hill (Books) Ltd., London Pp 63-72.

Hansen, H.J.M., A. G. Olsen and P.Rosenkilde, 1996. The effect of cu on gill and esophagus lipid metabolism in the rainbow trout (*oncorhynchus mykiss)* . *Comp.Biochem. Physiol.*, 113(1): 23-29.

Khangarot, B.S., Mathur, S. and Durve, V.S. 1980. Studies on the acute toxicity of copper on selected fresh water organism. *Science and Culture* . 47(12): 429-430.

Langston, W.J. 1990. Toxic effects of metals and the incidence of marine ecosystems. In: Furness, R.W., Rainbow, P.S. (Eds.), Heavy Metals in the Marine Environment. CRC Press, New York.

Lopes, P. A., Pinheriro, T., Santos, M.C., da Luz Mathias, M., Collares-Pereira, M.J., and A. M. iegas-Crespo, 2001. Response of antioxidant enzymes in freshwater fish populations (Leuciscus alburnoides complex) to inorganic pollutants exposure. *Sci, Total Environ.*, **280**: 153-63.

Mahajan, C.L. and Dheer, J.S. 1979. Cell types in the peripheral blood of an air-breathing fish, *Channa punctatus*. *J. Fish. Biol*., 14: 481-487.

Nammalwar, P., 1987. Environmental pollution problem in the coastal estuaries around Madras, India - A case study. In: Proceedings of the national conference on environmental impact on biosystem, January 1987, Loyola College, Chennai, pp. 157-162.

Newman, M.C. and Jagoe, C.H., 1994. Ligands and the bioavailability of metals in aquatic environments. In: Hamelink, J.L., Landrum, P.F., Bergman, H.L., Benson, W.H. (Eds.), Bioavailability: Physical, Chemical, and Biological Interactions. Lewis Publications, Boca Raton, FL, pp. 39-62.

Rana, S.V.S., Jayakumar, A. and Agarwal, R.P. 1982. Functional versus morphological changes in the liver after heavy metal poisoning in rats. Proceeding of all India symposium on physiological response of animal to pollution.

Rashed, M.N. 2001.Monitoring of environmental heavy metals in fish from Nasser Lake, *Environ. Int.* 27: 27–33.

Santhakumari, D. 1990. Effect of mercuric chloride on the histology of selected organs in the freshwater fish *Anabas testudineus* (Bloch) *M.phil. thesis*, Annamalai university, India.

Sehgal, R. and A.B. Saxena, 1986. Toxicity of zinc to a viviparous fish *Lebistes reticulates* (peters). *Bull. Environm. Contam. Toxicol.*, 36: 888-894.

Torres, P., L. Tort and R.Fish, 1987. Acute toxicity of copper to mediterranear dog fish. *Comp Biochem Physiol.*, 86: 169-171.

Tulasi, S.J., P. U. Reddy and J.V.Ramana Rao, 1992. Accumulation of lead and effects on total lipid derivatives in the freshwater fish *Anabus testudineus* (Bloch). *Ecotox. Environ. Safe*., 23:33-38.